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1998 Kemp's Ridley Sex Ratio Project
Rancho Nuevo, Mexico

GLADYS PORTER ZOO

Sea Grant
In Mississippi and Alabama

Progress Report to the Instituto Nacional de la Pesca

November 1998

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Progress Report to:

Dr. René Márquez-M.
National Sea Turtle Coordinator
Instituto Nacional de la Pesca
Manzanilla, Mexico

1998 Kemp's Ridley Sex Ratio Project

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1998 KEMP'S RIDLEY SEX RATIO STUDY

During 1998, a multi year study was initiated to implement state-of-the-art technology for monitoring hatching sex ratios in the Kemp's ridley recovery program. This sex ratio study represents a collaborative effort involving the Instituto Nacional de la Pesca (INP), Gladys Porter Zoo (GPZ, Brownsville, TX), University of Alabama at Birmingham (UAB), MS-AL Sea Grant Consortium, Universidad del Noreste (Tampico, Mexico), U.S. Fish and Wildlife Service, and the National Marine Fisheries Service. This study is being coordinated by Dr. René Márquez-M. (National Sea Turtle Coordinator, Instituto Nacional de la Pesca), Patrick Burchfield (U.S. Group Coordinator and Associate Director of the Gladys Porter Zoo) and Dr. Thane Wibbels (University of Alabama at Birmingham). The field component of this study is supported by personnel and funding from INP, GPZ, Universidad del Noreste, U.S. Fish and Wildlife Service, and the National Marine Fisheries Service. The sexing technology component of this study is supported through a three year grant (1998-2001) from MS-AL Sea Grant Consortium (Grant No. NA56RG0129).

Temperature-Dependent Sex Determination and the Kemp's Ridley

The Kemp's ridley, like all sea turtles, possesses temperature-dependent sex determination (Aguilar, 1987; Shaver et al., 1988; Wibbels et al., 1989). This aspect of turtle's biology has the potential of producing highly biased sex ratios, and a variety of past studies have suggested that, in some cases (e.g. strong male biases), this could decrease the effectiveness of nesting beach programs (Mrosovsky and Yntema, 1980; Morreale et al., 1982; Mrosovsky, 1983; Wibbels et al., 1989). Therefore, it would be optimal to monitor hatchling sex ratios in nesting beach programs.

Goals of the Kemp's Ridley Sex Ratio Study

The long term goal of the current project is to implement technology which will facilitate the monitoring of sex ratios produced in all future years of the Kemp's Ridley Recovery Program. The goals for Year 1 of this project are:

1) Develop and implement optimal methods for using miniature data loggers for monitoring sand and nest temperatures in the egg corrals at Rancho Nuevo, Tepehuajes, and Playa Dos.

2) Optimize methods for analyzing large volumes of temperature data generated from the miniature dataloggers.

3) Evaluate and optimize nonharmful blood sampling techniques for hatchlings Kemp's ridleys.

4) Evaluate the feasibility of obtaining relatively large numbers of blood samples for verifying sex ratios produced in individual nests.

5) Utilize hatchling blood samples to validate mullerian inhibiting hormone (MIH) sexing technique for use with Kemp's ridleys.

6) Use the MIH sexing technique to verify sex ratios from a sample of nests from which

hatchling blood samples and incubation temperatures were obtained during the 1998 nesting season.

7) Begin generating an "incubation temperature versus sex ratio" database.

8) Based on temperature and MIH sexing data, estimate sex ratios produced during the 1998 nesting season.

PROGRESS TO-DATE

Implementation of Temperature Data Loggers

The implementation of data loggers proceeded excellently during the 1998 nesting season. Two types of data loggers were used and evaluated: 1) "Hobo" data loggers which were enclosed in heat-sealed bags prior to use, and 2) "Optic Stowaways" which are sealed in a waterproof housing at the factory (Figure 1). Both types of dataloggers were effective in recording nest temperatures. The Optic Stowaways proved more convenient since they did not need to be sealed in bags and they required less space in the nest compared to a Hobo which had been sealed in a bag. All data loggers were programmed to record temperature at least once per hour. Daily temperature fluctuation within a nest or at midnest depth in the sand was relatively small (standard deviation was typically 1.0 °C or less, see Appendix A), therefore, recording temperatures at 1 hr intervals appears to be appropriate.

Data loggers were used to monitor sand and nest temperatures in egg corrals at Rancho Nuevo, Tepehuajes, and Playa Dos. Sand temperatures were monitored throughout the nesting season in all three corrals. This was accomplished by burying five dataloggers at midnest depth (approximately 30 to 35 cm) throughout each corral. These dataloggers were buried during April and recovered in late August. Data loggers were also placed directly into the approximate center of the egg clutches to monitor temperatures within individual nests in the egg corrals at Rancho Nuevo, Tepehuajes, and Playa Dos. During the 1998 nesting season, incubation temperature were monitored in 56 nests at Rancho Nuevo, 16 nests at Tepehuajes, and 17 nests at Playa Dos. The nests in which temperature was monitored were laid on dates ranging from early April to mid July (see Appendix A). Temperature variation within nests was investigated by placing data loggers at the top, middle, and bottom of the egg mass in several nests. Additionally, a fourth data logger was buried adjacent to each of these nests at mid nest depth, in order to compare sand temperature to nest temperature.

Analysis of Temperature Data

We have been analyzing the temperature data over the past several months. As indicated earlier, the typical daily temperature fluctuation within a nest or at midnest depth in the sand, was small (standard deviation typically less than 1.0 °C). Daily average temperatures and standard deviations were calculated for the data from all data loggers. The average sand temperatures at midnest depth (approximately 30 to 35 cm) throughout the 1998 nesting season in the egg corrals at Rancho Nuevo, Tepehuajes, and Playa Dos are shown in Figures 2, 3, and 4. In general, sand temperatures in all three egg corrals were similar. Early in the nesting season, sand temperatures were relatively cool (e.g. approximately 28 °C), but temperatures rose to approximately 30 °C by mid May and remained above that level for the remainder of the nesting season.

Examples of temperature variation within individual nests (top versus middle versus bottom of nest) are shown in Figure 5 and 6. During the first two thirds of the total incubation period, the temperatures within the nest varied by a maximum of approximately 1.0 °C. During the last third of incubation, the temperature in the middle of the egg mass was higher than regions at the top or bottom of the egg mass (presumably due to metabolic heat). Sand temperatures at midnest depth adjacent to the nest was typically within 1.0 °C or less of the temperatures within the nest.

Temperature data from individual nests are listed in Appendix A. Daily temperature averages and standard deviations were calculated for all data. The total incubation time was divided up into thirds, and the average temperature during the middle third of incubation (i.e. the thermosensitive period of sex determination) is listed along with minimum and maximum temperatures during the middle third of incubation (see Appendix A). Examples of temperatures within the nests are shown in Figures 6, 7, 8, 9, 10, 11, 12. Nests laid in mid April had incubation temperatures of approximately 28 to 30 °C during the middle third of incubation. Nests laid during late April had incubation temperatures of approximately 29 to 31 °C during the middle third of incubation, and nests laid from mid May through mid June had incubation temperature ranging from approximately 31 to 34 °C during the middle third of incubation.

Preliminary Prediction of Sex Ratios Based on Incubation Temperature

Previous studies by Aguilar, 1987, and Shaver et al., 1988, provide estimates of the effects of specific temperatures on sex determination in the Kemp's ridley. However, these data should be considered general estimates since factors such as clutch effects (Mrosovsky, 1988; Etchberger et al., 1991; Ewert et al., 1994; Lang and Andrews, 1994) can significantly affect sex ratios. Regardless, the data from those studies suggest a pivotal temperature near 30° C (temperature producing a 1:1 sex ratio) and that temperatures of approximately 31 °C or greater produce most or all females. Using those data as a reference, the temperature data from the 1998 nesting season suggests that both males and females were produced. Nests laid early in the nesting season (April) would be predicted to produce males or both males and females, whereas, nests laid during or after mid May would be predicted to produce most if not all females. Overall, the temperature data suggests the possibility of a female bias. However, the temperature data needs to be compared with the chronology of egg production during the nesting season and with the results from the MIH sexing technique before accurate conclusions can be drawn.

Evaluation of Blood Sampling Methodology

Blood sampling techniques were evaluated for hatchling Kemp's ridleys. The optimal sampling location was determined to be directly under the nuchal scute (Figure 13). Using this location, blood samples were consistently obtained from hatchlings. All hatchlings appeared very active after the blood sampling and no adverse effects were detected. A 1.0 cc syringe with a 25 gauge needle was used to take a small volume of blood (approximately 50 ul). Syringes were heparinzed prior to use, and the neck of each hatchling was swabbed with 70% ethanol prior to sampling. Blood samples were transferred to 0.5 ml microcentrifuge tubes and spun to separate the blood cells from the plasma. The plasma was pipetted into cryotubes and frozen in liquid nitrogen.

Implementation of Blood Sampling

The blood sampling methodology proved to be a practical means of obtaining relatively large numbers of blood samples from hatchling Kemp's ridleys. The biologists at Rancho Nuevo became experts at obtaining and processing hatchling blood samples (Figures 14, 15, 16, 17). Blood samples were obtained from hatchlings from 33 nests (see appendix B). In most cases, 10 hatchlings were sampled from each nest. The samples were obtained from nests which had received data loggers for monitoring incubation temperature.

Blood Sample Transport, Storage, and Analysis

All blood samples have been transported from Rancho Nuevo to UAB (CITES export permit MEX 06082 and CITES import permit US 841026). Samples are currently being stored at -196 °C in liquid nitrogen which insures that they will not degrade. We are almost finished with our analysis of the temperature data, so we will be validating our MIH assay for use with Kemp's ridley samples and then assaying all samples over the next several months (see below).

MIH (Mullerian Inhibiting Hormone) Sexing Technique Validation

We have developed an assay for mullerian inhibiting hormone (MIH, also called antimullerian hormone or mullerian inhibiting substance) which we will use as a sexing technique for hatchling Kemp's ridleys. In vertebrates, both male and female embryos develop mullerian ducts which form the oviducts in females (e.g. fallopian tubes and uterus). Male vertebrates begin producing MIH during late embryonic development and this hormone stimulates the degeneration of the mullerian ducts. Previous studies indicate that MIH levels are very high in young males, but not in females. For example, in humans, MIH in males is extremely high at birth and remains high for six years or more, whereas levels are very low or nondetectable in females.

We have previously 1) cloned the cDNA for turtle MIH (Figure 18), 2) produced recombinant turtle MIH protein (Figure 19), 3) identified several antisera which crossreact with turtle MIH, and 4) developed enzyme-linked immunosorbent assays for turtle MIH (Figure 20). We have been very conservative in our development of the MIH assay. We want to stockpile enough antisera and MIH standards to that we can use the exact same assay for many future years of the Kemp's ridley program. Additionally, we are trying different combinations of antisera in the assays in order to generate an optimal assay with maximal sensitivity prior to our validation experiments with the Kemp's ridley samples. Over the next several months we will be optimizing our assay for turtle MIH, validating it for use with Kemp's ridleys samples, and then assaying all samples collected during the 1998 nesting season (see Appendix B).

Collaborative Nature of This Research

The current project would not be possible without the collaboration of a wide variety of agencies and individuals. The sex ratio project is being coordinated by Dr. René Márquez-M. of the Instituto Nacional de la Pesca (Figure 21), Patrick Burchfield of the Gladys Porter Zoo (Figure 21), and Dr. Thane Wibbels of the University of Alabama at Birmingham. The sex ratio research on the nesting beach at Rancho Nuevo was supervised and coordinated during the 1998 nesting season by Manuel Garduno, Juan Diaz, Alma Leo Perido, and Jaime Pena. Manuel Garduno (Figure 22) helped facilitate obtaining the CITES export permit for the blood samples. Jaime Pena (Figure 22) was invaluable in helping coordinate Dr. Wibbels' six trips to Mexico over the 1998 nesting season. A large number of researchers at the nesting beach significantly contributed to the success of the sex ratio project during the 1998 nesting season (Figure 23 and 24). I would like to acknowledge the following individuals for the contributions to the sex ratio project on the nesting beach:

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Gustavo Andrade Rubio
Erick Gonzalez Salem

Sheryl Anne O'Shea
Shelly Denise Scroggs
Janina Jakubowska
Ramon Silva Arizabalo
Jaime Ortiz
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Maria Elena Romero Dey
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Rene Messier
Isadore Toledo

The field component of this study is supported by personnel and funding from INP, GPZ, Universidad del Noreste, U.S. Fish and Wildlife Service, and the National Marine Fisheries Service. The sexing technology component of this study is supported through a three year grant (1998-2001) to Dr. Wibbels from MS-AL Sea Grant Consortium (Grant No. NA56RG0129).

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List of Figures

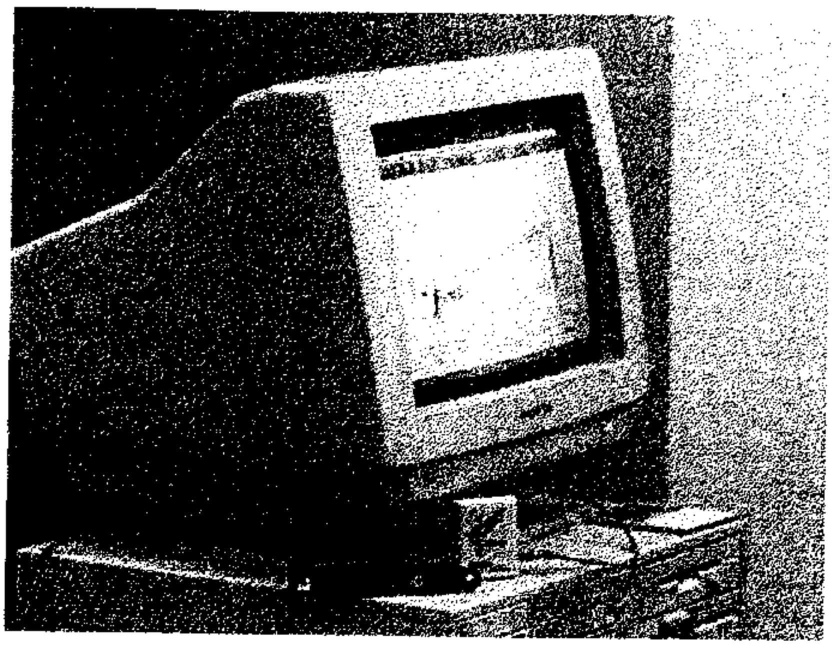


Figure 1. The two types of temperature data loggers utilized during the 1998 nesting season are shown in front of the computer monitor. An "Optic Stowaway" is shown on the left and a "Hobo" is shown on the right (attached to the computer by a cable). Both types are capable of recording and storing several thousand temperature recordings. The Hobo is enclosed in a heat-sealed bag prior to use, while the Optic Stowaway is sealed in a waterproof housing at the factory.

Figure 2. Sand Temperatures at Rancho Nuevo Egg Corral Throughout the 1998 Nesting Season

Each line represents an individual data logger

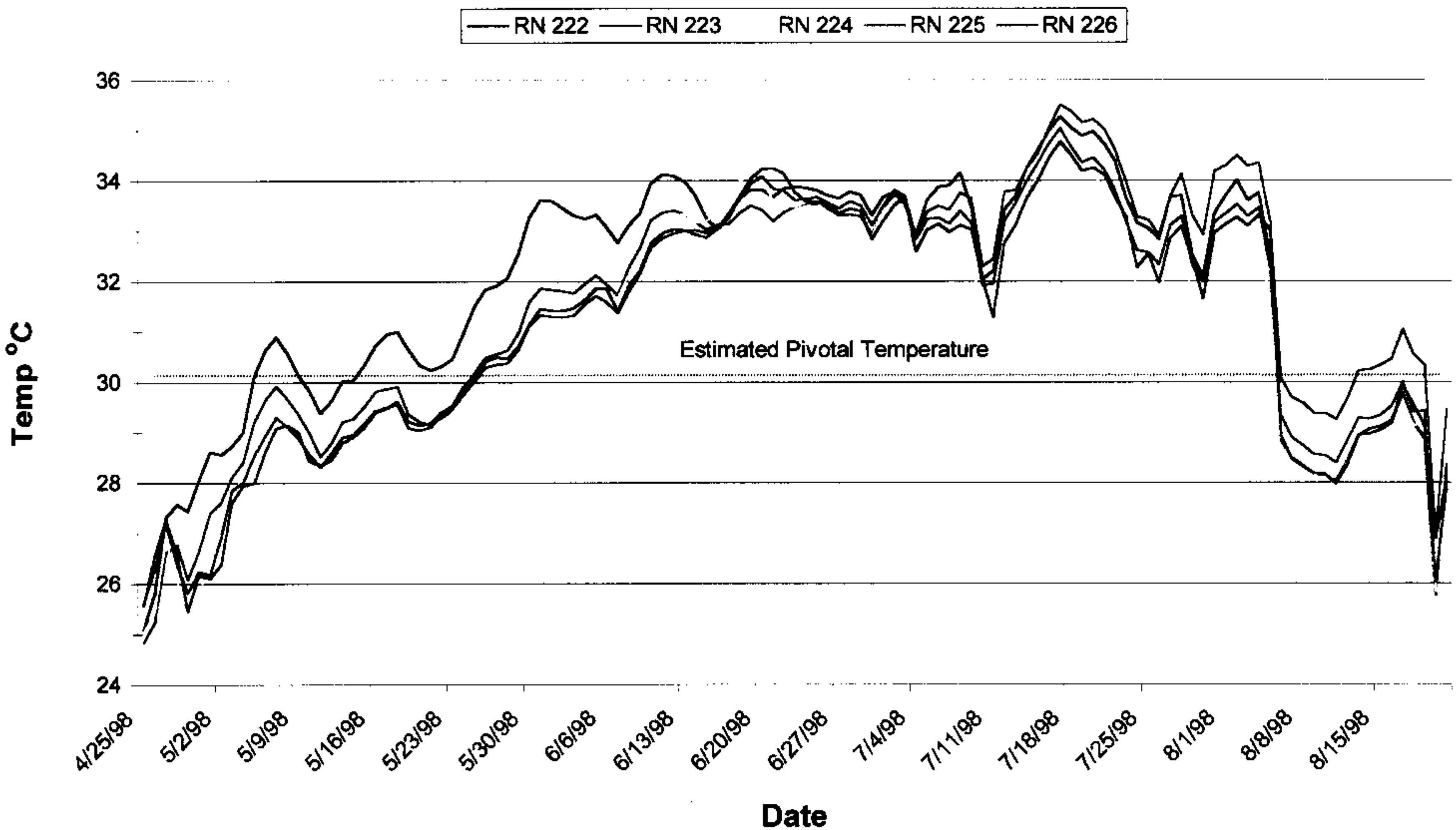


Figure 3. Sand Temperatures at Tepehuajes Egg Corral Throughout the 1998 Nesting Season

Each line represents an individual data logger

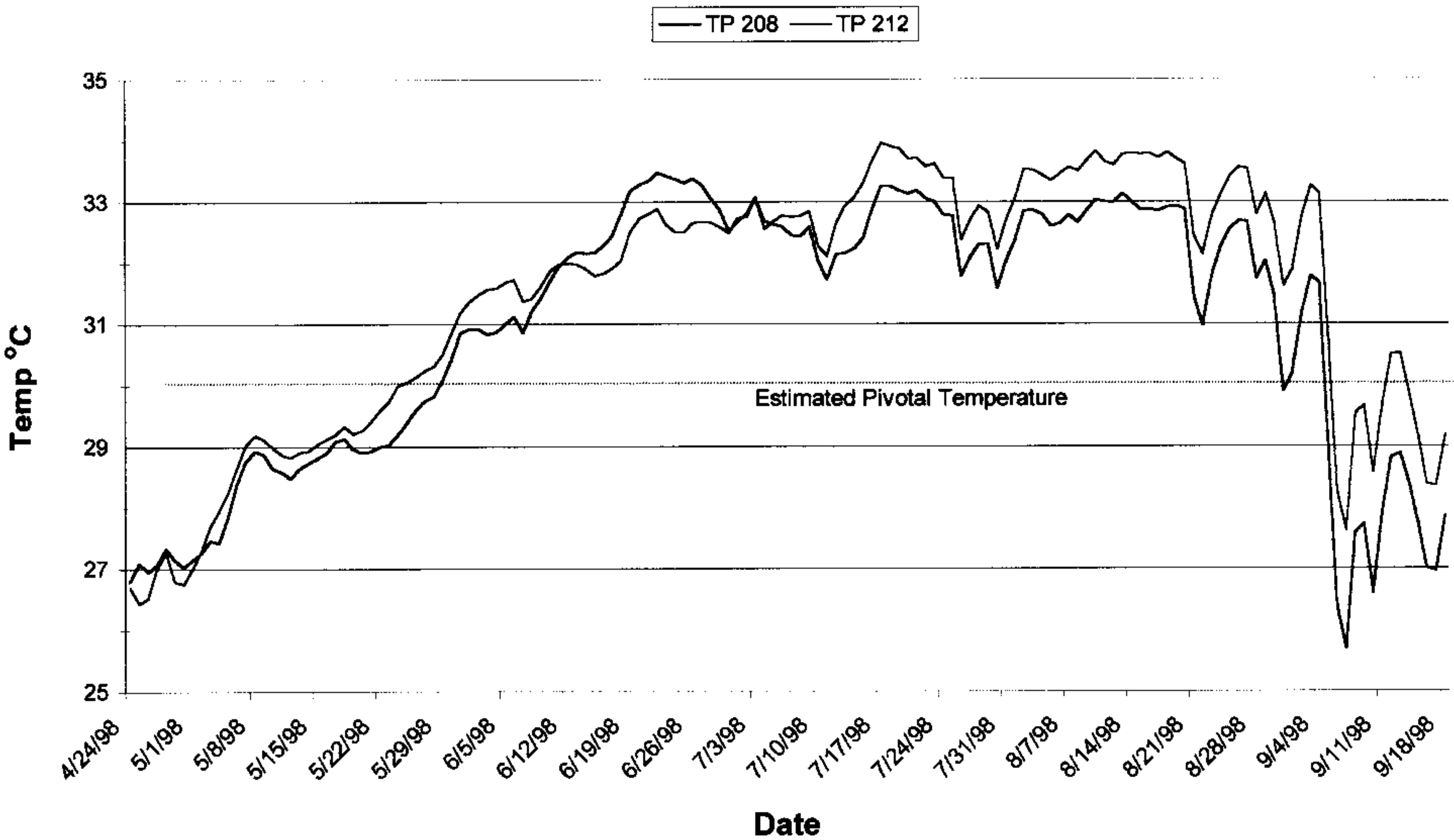


Figure 4. Sand Temperatures at Playa Dos Egg Corral Throughout the 1998 Nesting Season

Each line represents and individual data logger

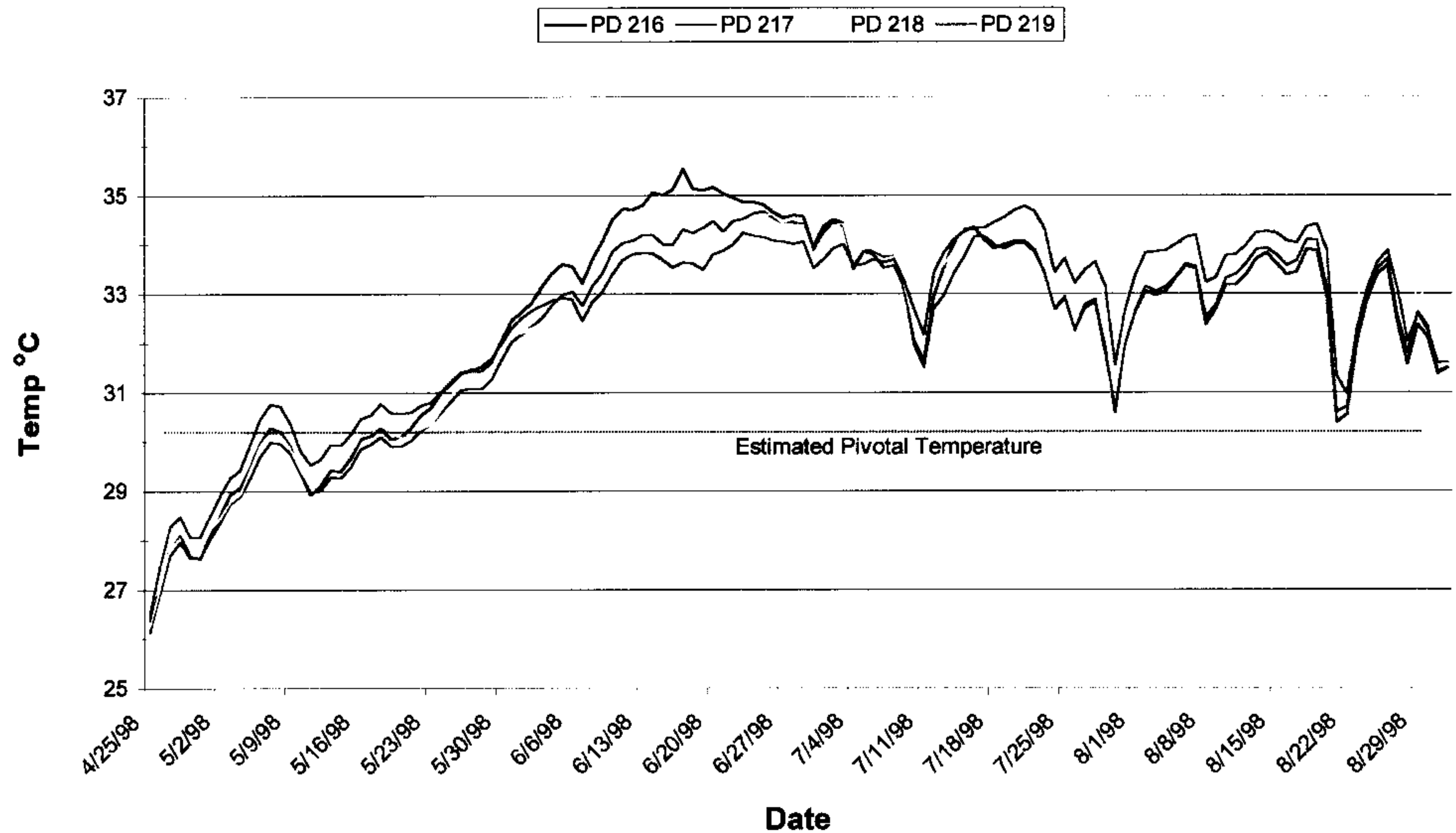


Figure 5. Temperature Variation Within Nest 1604
Data Loggers were placed at the Top, Middle, Bottom, and Adjacent to the nest

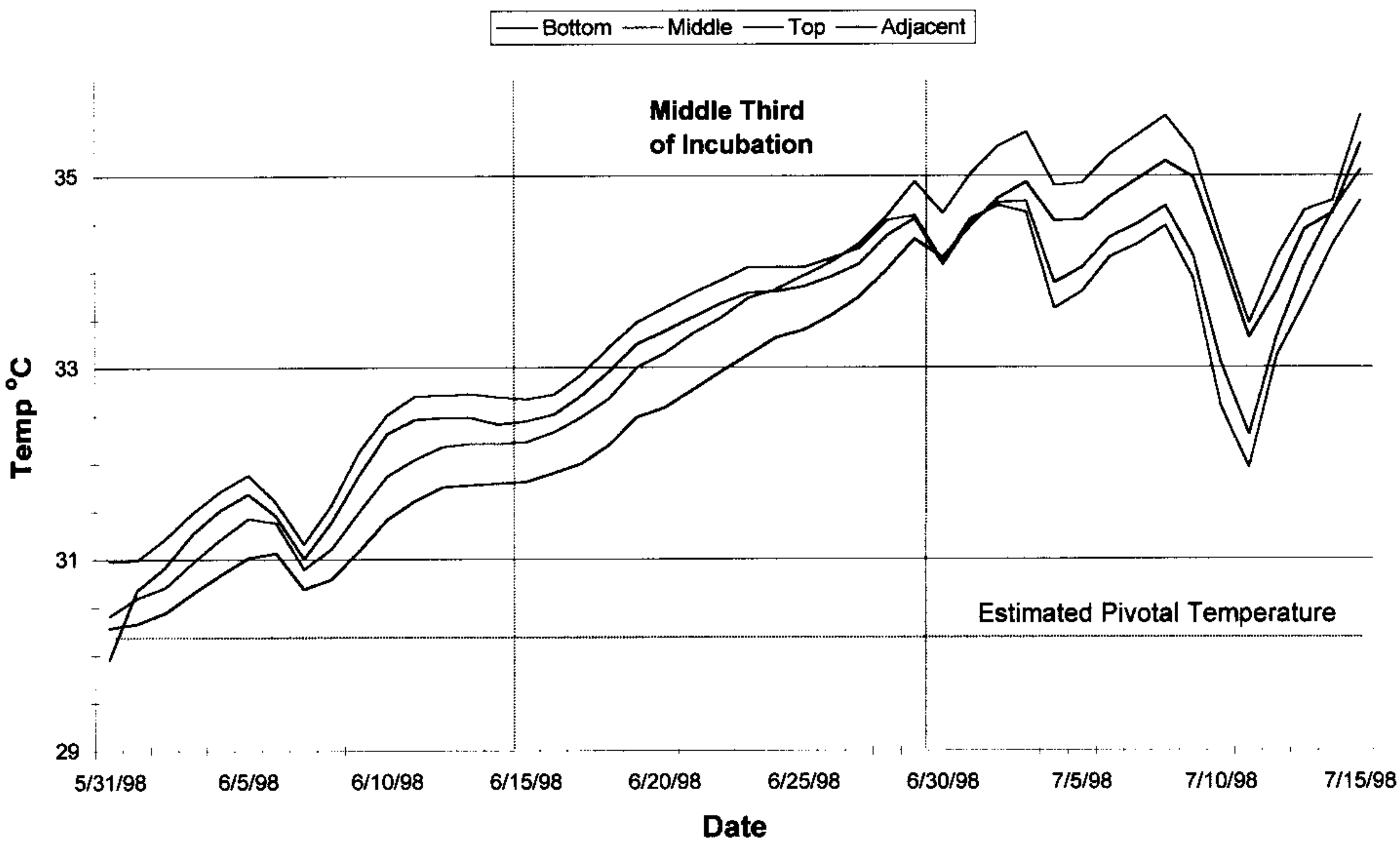


Figure 6. Temperature Variation Within Nest 1605

Data Loggers were placed at the Top, Middle, Bottom, and Adjacent to the nest

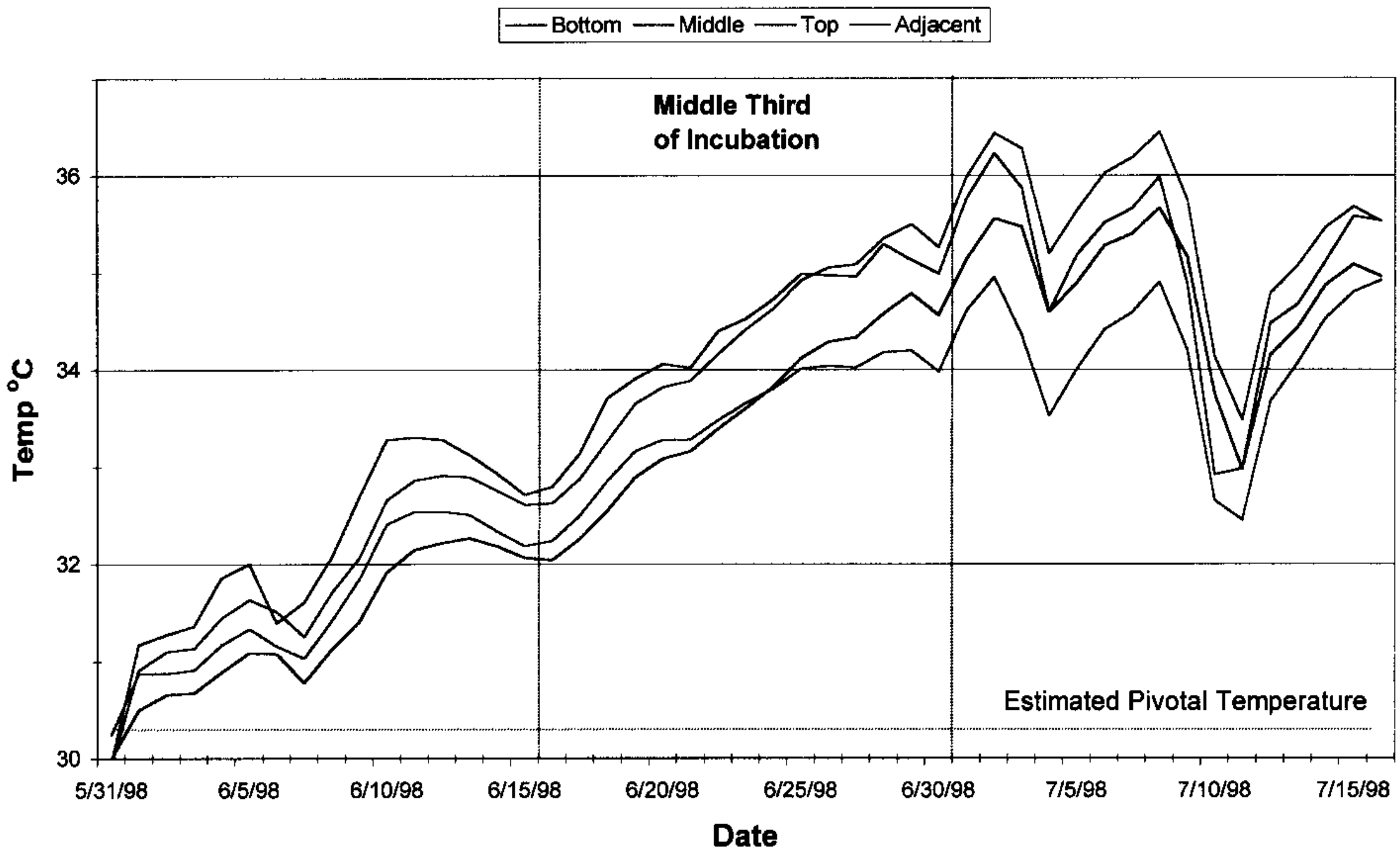


Figure 7. Examples of Nest Incubation Temperatures
Mid April Lay Dates
Rancho Nuevo 1998
Temperatures Within Individual Nests. Each Line Represents A Single Nest

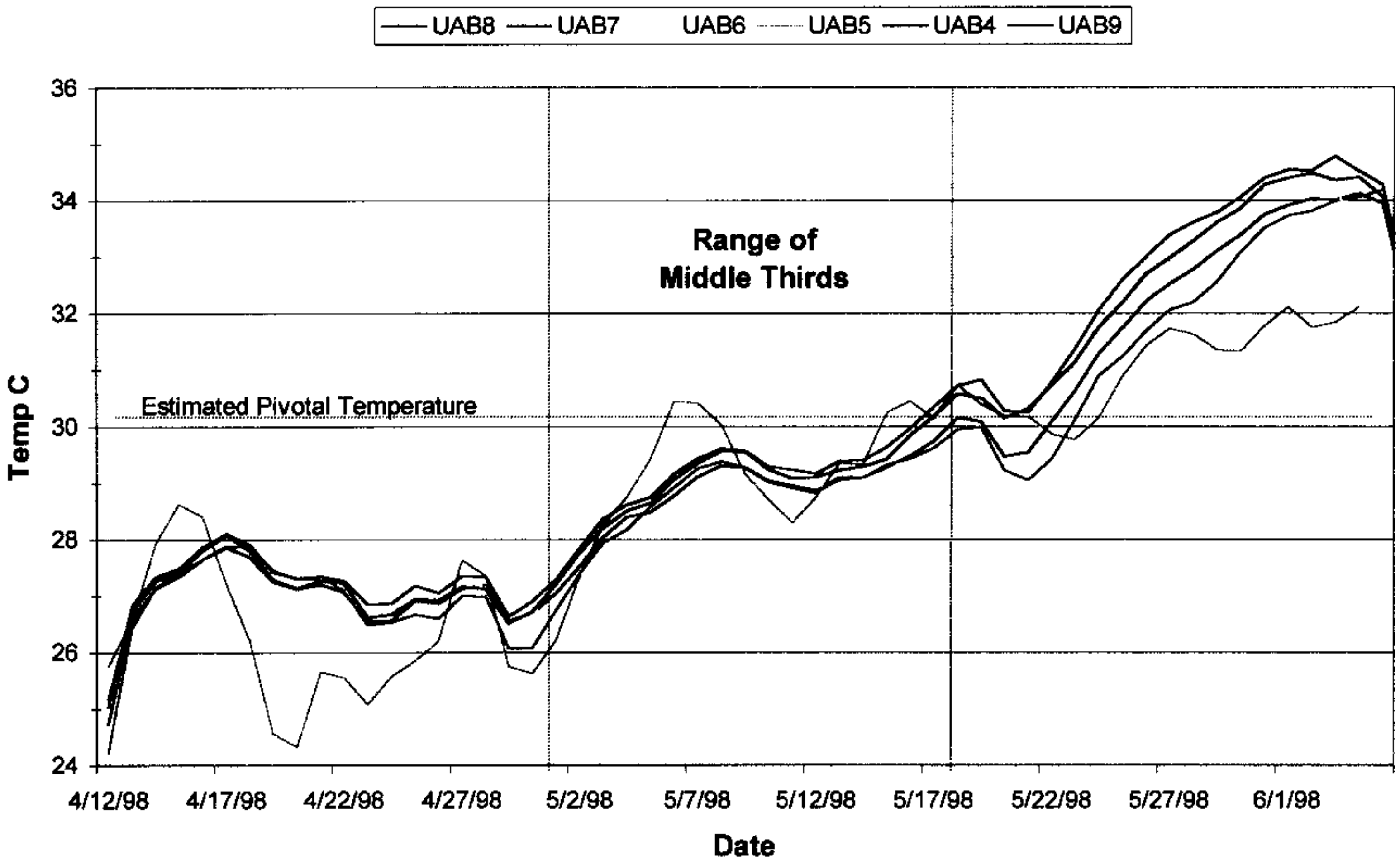


Figure 8. Examples of Nest Incubation Temperatures
Late April Lay Dates
Rancho Nuevo 1998
Temperatures Within Nests. Each Line Represents A Single Nest

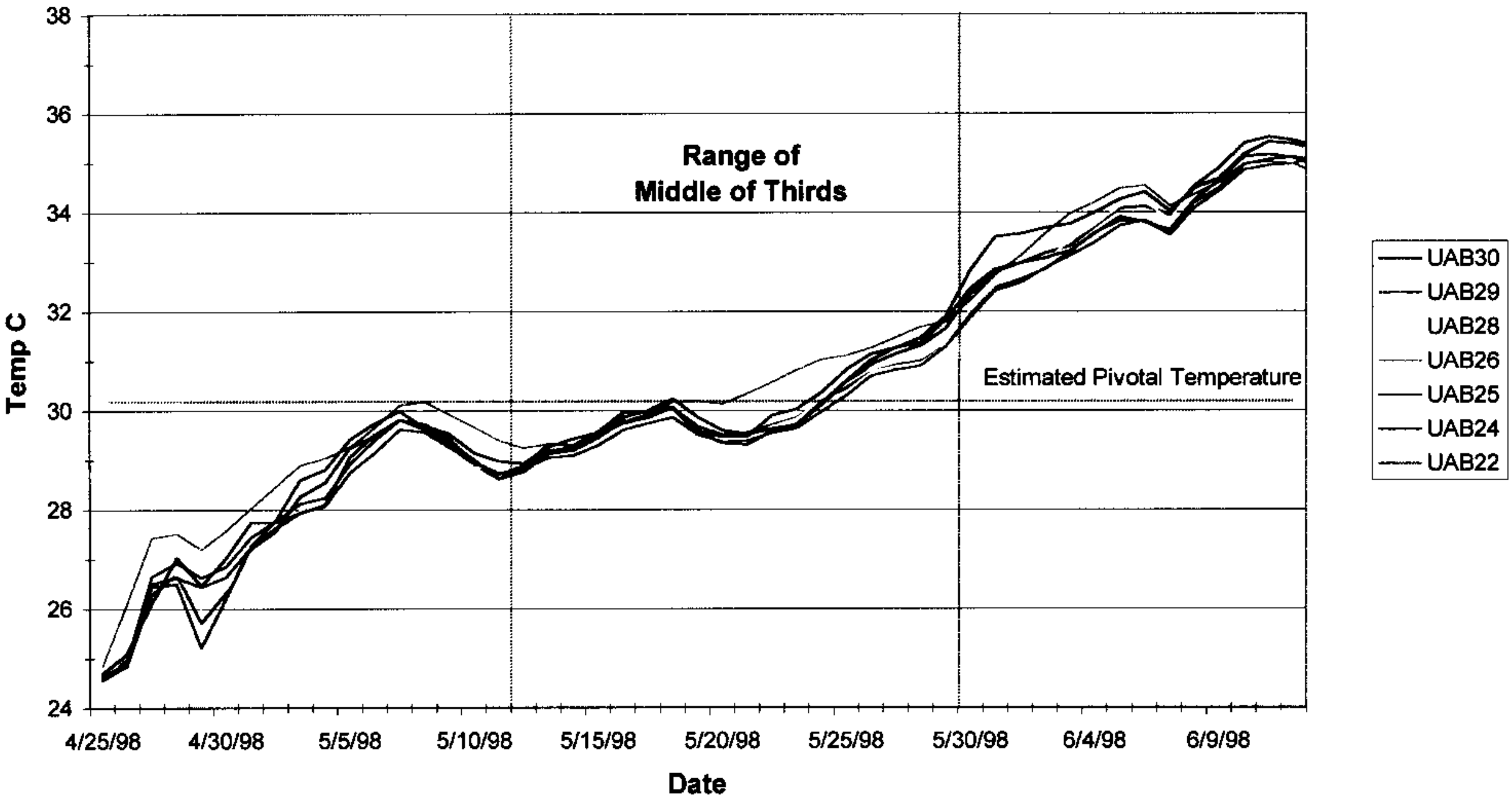


Figure 9. Examples of Nest Incubation Temperatures
Mid-May Lay Dates
Rancho Nuevo 1998
Temperatures Within Individual Nests. Each Line Represents A Single Nest

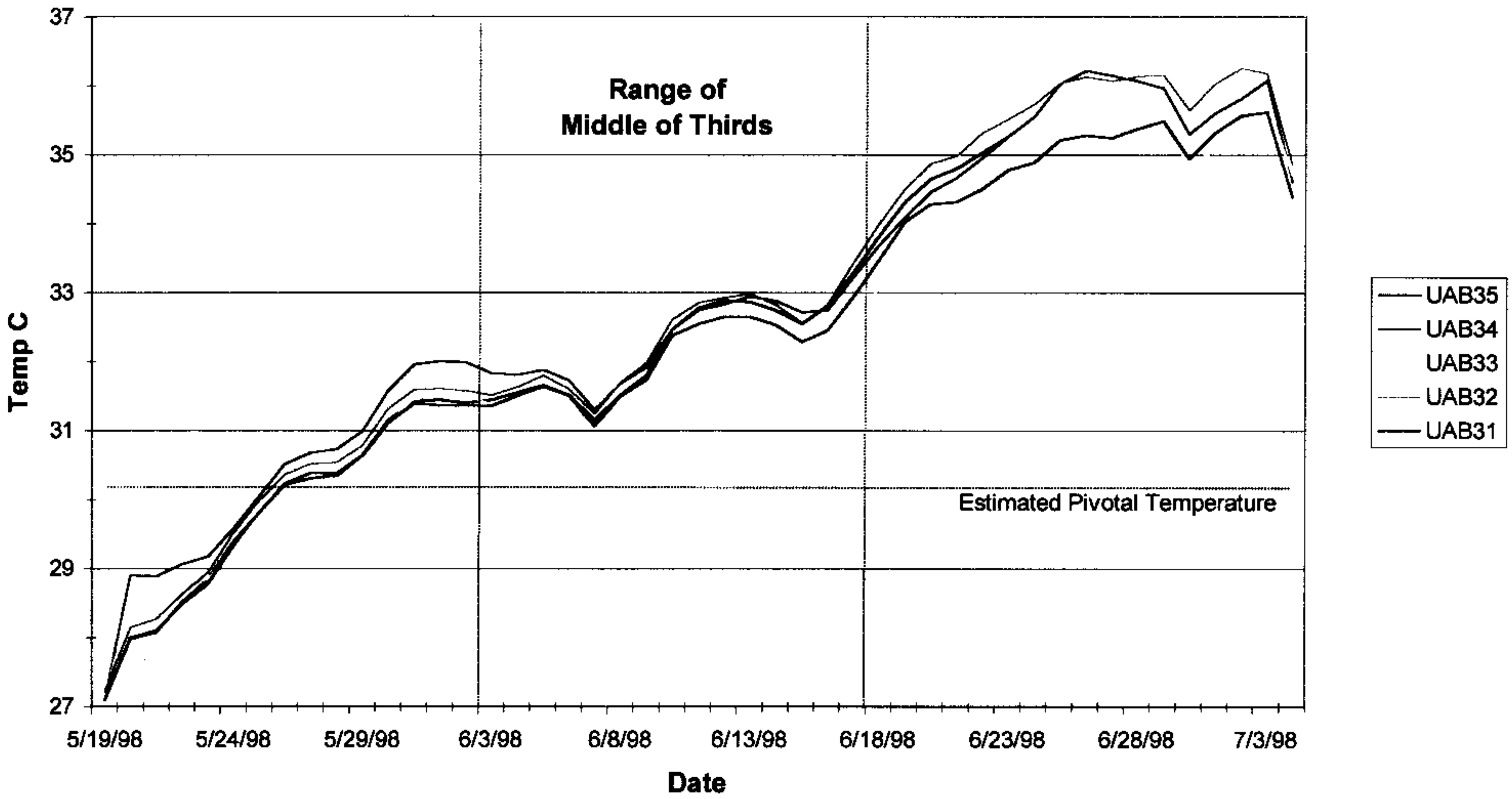


Figure 10. Examples of Nest Incubation Temperatures
Late May Lay Dates

Rancho Nuevo 1998

Temperatures Within Individual Nests. Each Line Represents A Single Nest

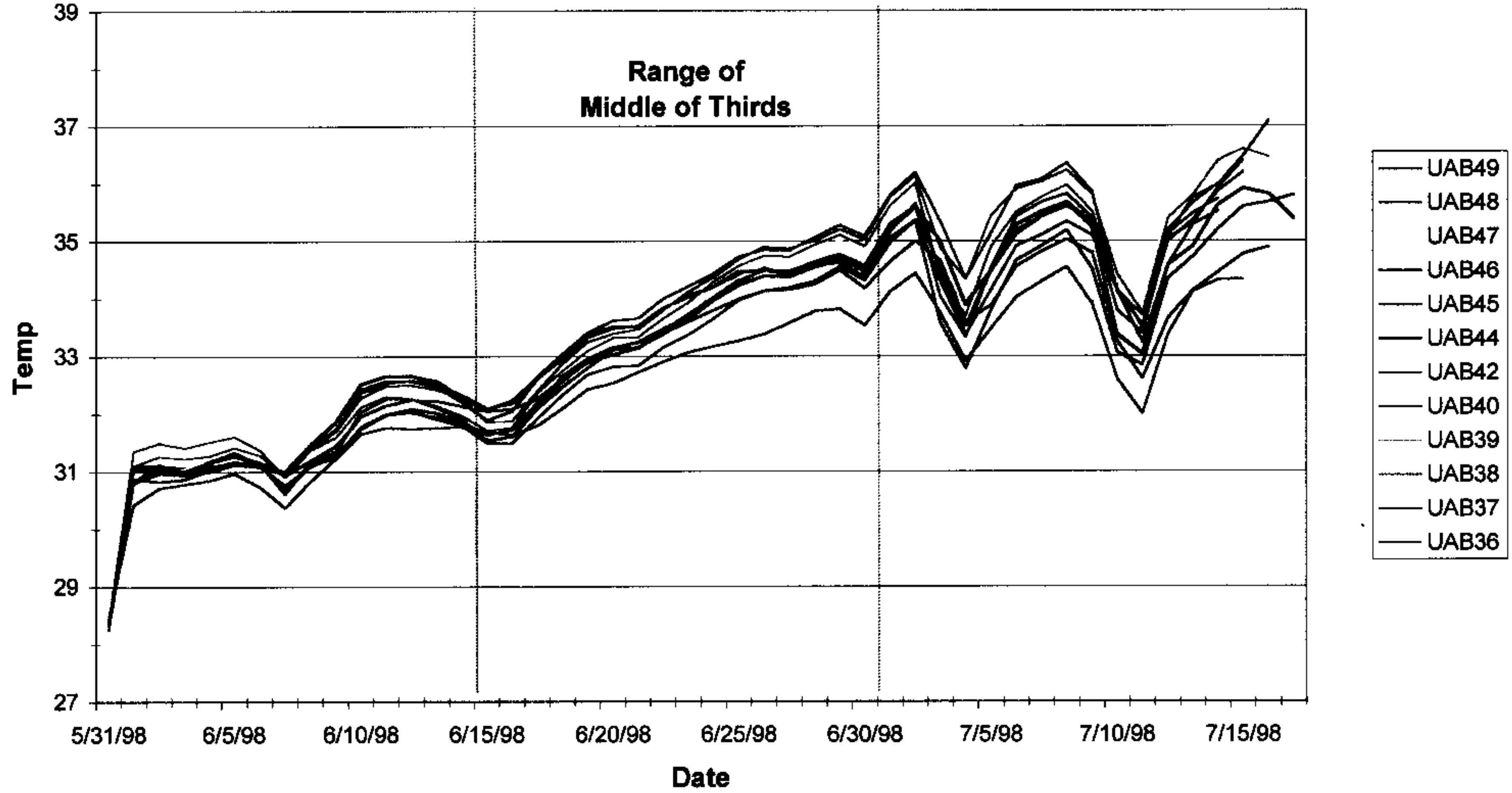


Figure 11. Examples of Nest Incuabtion Temperatures

Mid June Lay Dates

Tepehuajes 1998

Temperatures Within Individual Nests

Each Line Represents A Single Nest

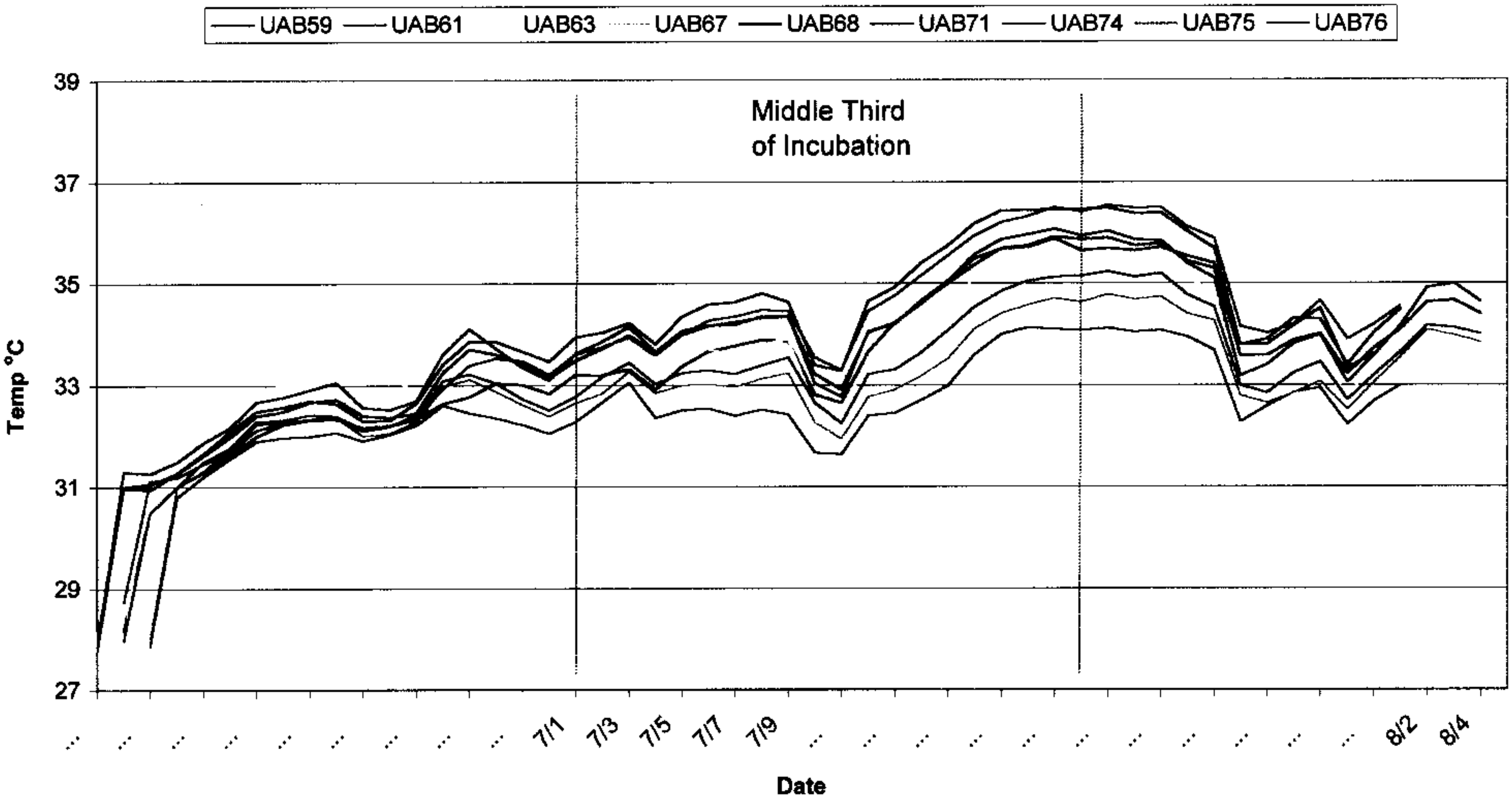


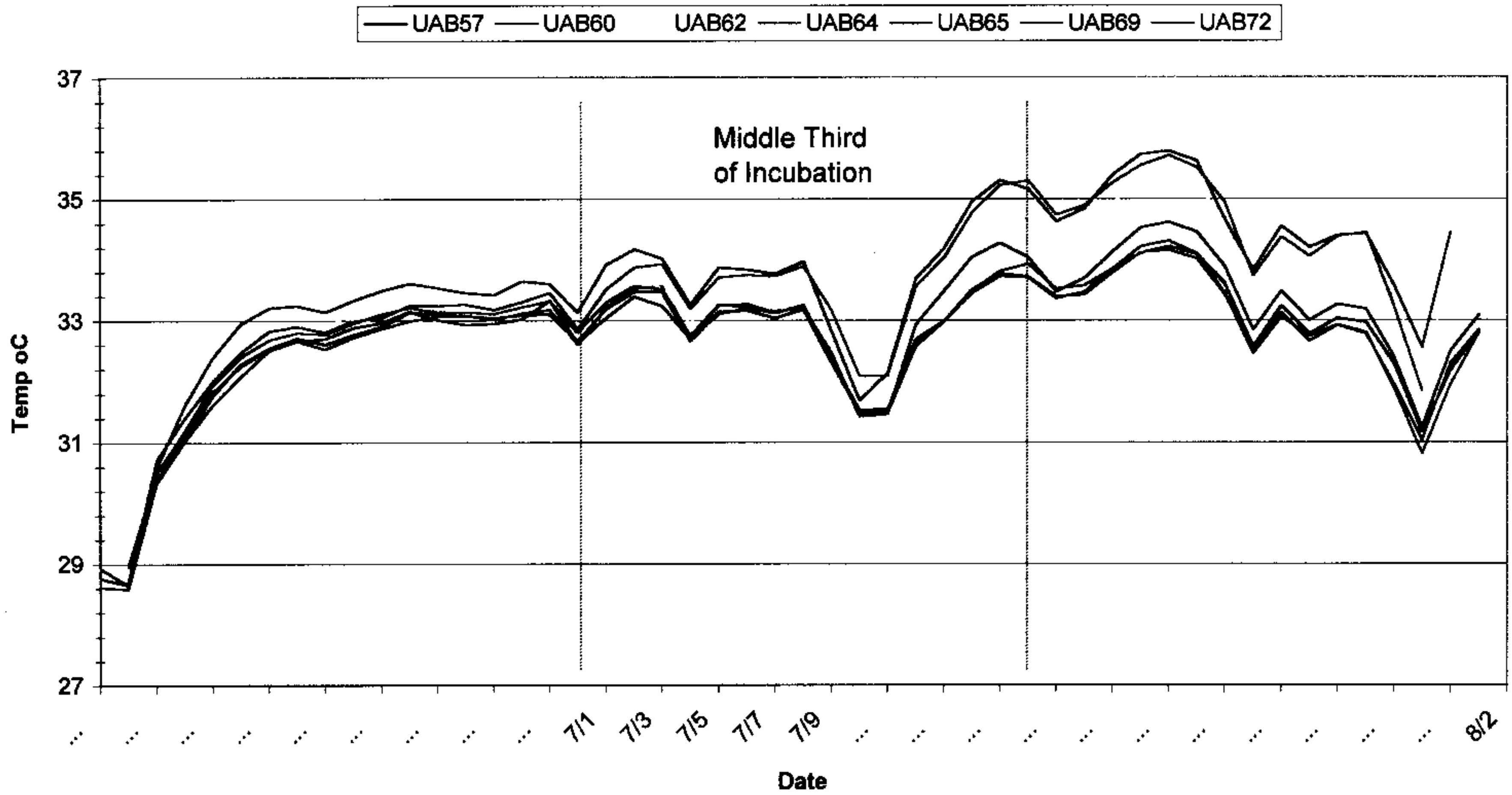
Figure 12. Examples of Nest Incubation Temperatures

Mid June lay Dates

Playa Dos 1998

Temperatures Within Individual Nests

Each Line Represents A Single Nest



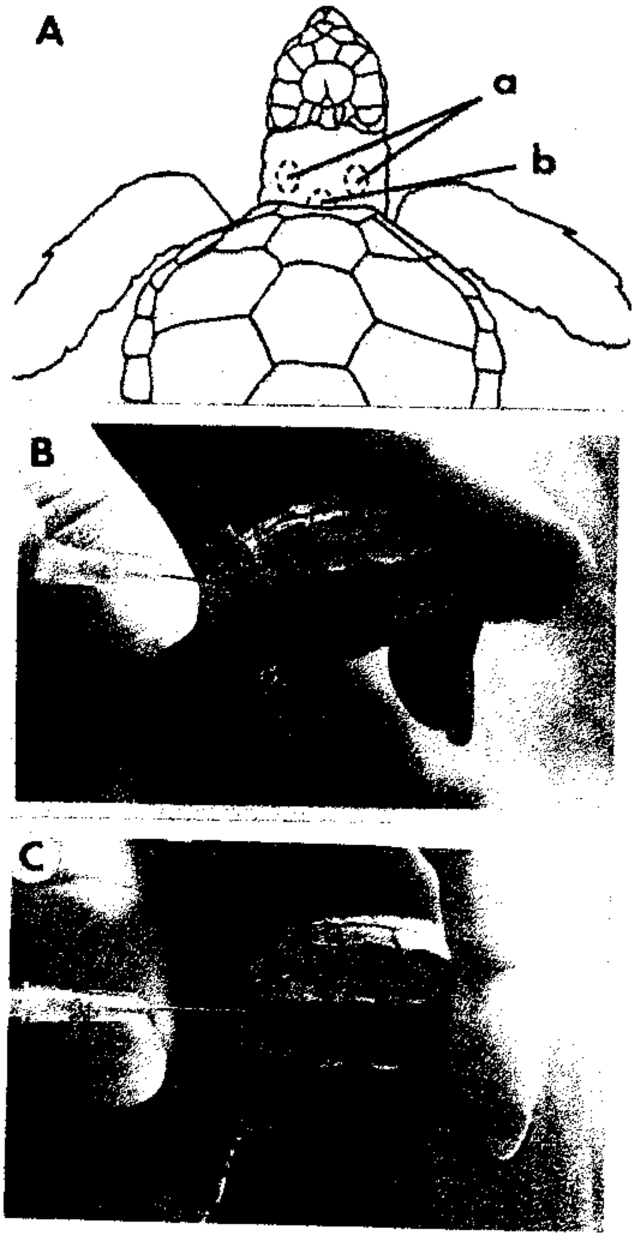


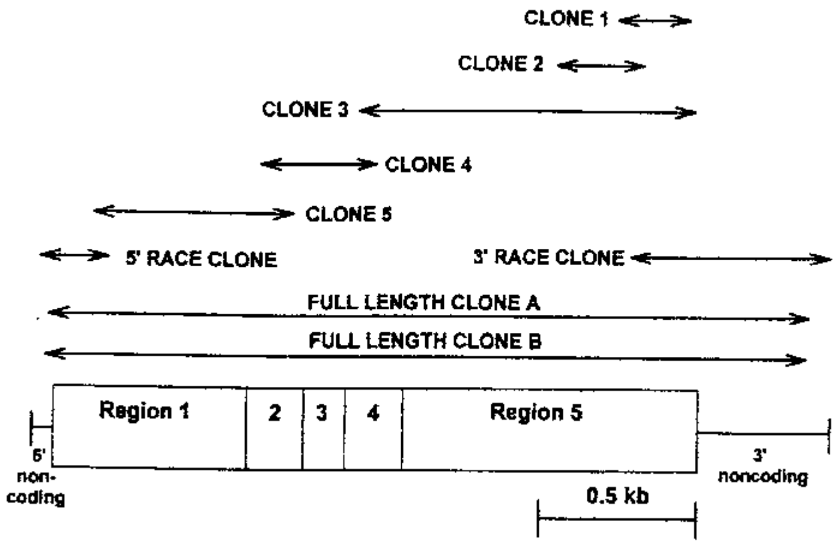
Figure 13. Several blood sampling locations were evaluated. The location shown in "C" (just under the nuchal scute) proved to be the optimal location for obtaining blood samples from hatchling Kemp's ridleys. This method of blood sampling appears to be a practical means of obtaining samples from large numbers of hatchlings. No adverse effects were detected.

Figure 14. The researchers at Rancho Nuevo became experts at obtaining blood samples from hatchlings. In the above photo (left to right) Gustavo Vazquez, Ana L. Cruz, Manuel Garduno, Mario Sandoval, Alfredo Salgado, Alma Leo Peredo, and Juan Diaz are shown prepping hatchlings and taking hatchling blood samples.

Figure 15. (Left to right) Ana L. Cruz, Ramon Silva, and Thane Wibbels are shown obtaining hatchling blood samples. The sampling methods were shown to be a practical means of obtaining relatively large numbers of blood samples.

Figure 16. (Left to right) Alfredo Salgado, Mario Sandoval, and Ana L. Cruz are shown cleaning the necks of hatchlings with 70% ethanol prior to taking blood samples.

Figure 17. (Left to right) Alma Leo Peredo, Mario Sandoval, and Ana L. Cruz are shown processing the blood samples.



PCR Cloning of Turtle MIH cDNA

Figure 18. In order to develop an assay for turtle mullerian inhibiting hormone (i.e. MIH), a polymerase chain reaction strategy was used to clone the entire turtle MIH cDNA . MIH is produced by male vertebrates and it causes the degeneration of the mullerian ducts (i.e. the embryonic oviducts).

Figure 19. To produce the assay for MIH, purified turtle MIH was needed. We inserted turtle MIH cDNA into an pET expression vector in order to produce and purify recombinant turtle MIH protein. The pET expression system attaches a His-Tag onto the expressed protein so that it can be purified on a His-Tag column. Lane A shows molecular weight markers. Lane B shows crude cell extract prior to expression and purification. Lane C shows a single band (i.e. MIH) after expression and column purification.

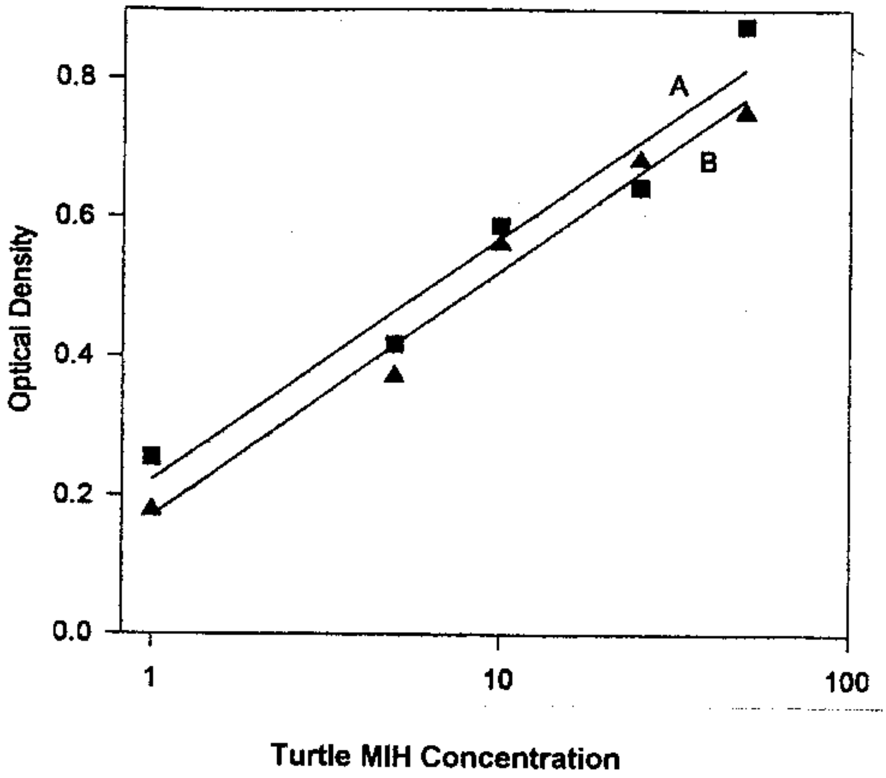


Figure 20. Turtle MIH Assays: We have identified two different antisera which are specific for turtle MIH. The diagram above shows the standard curves for two enzyme-linked immunosorbent assays (ELISAs)for turtle MIH that we have developed. We are currently optimizing sensitivity by utilizing both antisera simultaneously in a single assay. Over the next several months we will be validating the use of the assay for accurately sexing Kemp's ridleys.

Figure 21. The sex ratio project is being coordinated by Dr. Réne Márquez-M (shown on the left) of the Instituto Nacional de la Pesca, Patrick Burchfield (shown on the right) of the Gladys Porter Zoo, and Dr. Thane Wibbels (not shown) of the University of Alabama at Birmingham.

Figure 22. Manuel Garduno (shown on left), Juan Diaz (not shown), Alma Leo Peredo (not shown), and Jaime Peña (shown on right) coordinated the sex ratio research on the nesting beach during 1998. Manuel Garduno facilitated the completion of the CITES export permits for the blood samples. Jaime Peña was invaluable in helping coordinate numerous trips to Rancho Nuevo by Dr. Wibbels.

Figure 23. The sex ratio project would not have been possible without the help of numerous individuals at the nesting beach during the 1998 nesting season.

Figure 24. As part of the 1998 sex ratio project, biologists at Rancho Nuevo evaluated microscopic methods for determining the sex of hatchlings which are found dead in the nests.

APPENDIX A

Incubation Temperature Data from Nests during the 1998 Kemp's Ridley Sex Ratio Project

Data loggers were placed in the center of the egg mass. Temperatures were recorded at least once per hour over the entire incubation period. Daily average temperatures and standard deviations were calculated for all data sets, and these data were used to calculate average temperatures during the thermosensitive period (i.e. the middle third of incubation). Previous studies suggest that Kemp's ridleys have a pivotal temperature (temperature producing a 1:1 sex ratio) of approximately 30 °C (Aguilar, 1987; Shaver et al., 1988).

Legend For Data Spreadsheet:

Inc.Duration = total duration of incubation in days

Location: RN= Rancho Nuevo, PD = Playa Dos, TP = Tepehuajes

Mid 1/3 Dates = dates representing the middle third of incubation which approximates the period when sex determination is sensitive to temperature.

Max T Mid 1/3 = Maximum temperature recorded in the nest during the middle third of incubation

Min T Mid 1/3 = Minimum temperature recorded in the nest during the middle third of incubation

Avg T Mid 1/3 = Average temperature recorded in the nest during the middle third of incubation

SD = Average standard deviation of daily temperatures during the middle third of incubation

LayDate	Nest #	Location	Data Logger	HatchDate	Inc.Duration	Mid 1/3 Dates	MaxT Mid1/3	MinT Mid1/3	AvgT Mid1/3	SD
4/6/98	m27	RN	UAB 10	6/9/98	65 days	4/28/98-5/18/98	29.4	26.8	27.2	0.822
4/6/98	m26	RN	UAB 11	6/6/98	62 days	4/27/98-5/16/98	29.6	26.3	28.4	1.033
4/6/98	m25	RN	UAB 12	6/4/98	60 days	4/26/98-5/15/98	31.0	25.7	28.6	1.654
4/11/98	m28	RN	UAB 5	6/7/98	58 days	4/30/98-5/19/98	30.7	25.6	29.1	1.400
4/12/98	m33	RN	UAB 4	6/6/98	56 days	5/1/98-5/18/98	30.7	27.3	29.2	0.820
4/12/98	m32	RN	UAB 6	6/4/98	54 days	4/30/98-5/17/98	30.2	26.7	28.9	0.920
4/12/98	m237	RN	UAB 7	6/6/98	56 days	5/1/98-5/18/98	29.9	26.8	28.8	0.800
4/12/98	m34	RN	UAB 8	6/6/98	56 days	5/1/98-5/18/98	30.2	27.1	28.9	0.750
4/12/98	m31	RN	UAB 9	6/6/98	56 days	5/1/98-5/18/98	30.6	27.2	29.1	0.812
4/12/98	m248	RN	UAB 14	6/7/98	57 days	5/1/98-5/19/98	31.5	26.3	29.5	1.335
4/24/98		RN	#198	6/15/98	53 days	5/12/98-5/28/98	30.8	28.8	29.7	0.588
4/24/98		RN	#199	6/14/98	52 days	5/11/98-5/28/98	32.1	29.1	30.3	0.885
4/24/98	m481	RN	#200	6/15/98	53 days	5/12/98-5/28/98	30.9	28.9	29.8	0.597
4/24/98	m491	RN	#201	6/15/98	53 days	5/12/98-5/28/98	31.5	29.1	30.2	0.743
4/24/98		RN	#202	6/12/98	50 days	5/11/98-5/26/98	31.3	29.2	30.1	0.642
4/24/98	m471	RN	#203	6/15/98	53 days	5/12/98-5/28/98	31.3	28.7	29.8	0.757
4/24/98	m495	RN	#204	6/15/98	53 days	5/12/98-5/28/98	31.2	28.7	29.8	0.750
4/24/98	m486	RN	#205	6/15/98	53 days	5/12/98-5/28/98	31.4	29.1	30.2	0.698
4/24/98	m474	RN	#206	6/15/98	53 days	5/12/98-5/28/98	31.2	28.8	29.9	0.700
4/24/98	m480	RN	#207	6/14/98	52 days	5/11/98-5/28/98	32.1	29.2	30.4	0.914
4/25/98	m588	RN	UAB 20	6/15/98	52 days	5/12/98-5/29/98	31.3	28.7	29.8	0.755
4/25/98	m576	RN	UAB 22	6/14/98	51 days	5/12/98-5/28/98	30.9	28.8	29.7	0.618
4/25/98	m597	RN	UAB 23	6/13/98	50 days	5/12/98-5/27/98	32.1	29.4	30.5	0.736
4/25/98	m595	RN	UAB 24	6/15/98	52 days	5/12/98-5/29/98	31.9	28.9	30.1	0.823
4/25/98	m589	RN	UAB 25	6/16/98	53 days	5/13/98-5/29/98	31.8	29.3	30.2	0.796
4/25/98	m599	RN	UAB 26	6/15/98	52 days	5/12/98-5/29/98	31.8	29.3	30.4	0.834
4/25/98	m580	RN	UAB 28	6/15/98	52 days	5/12/98-5/29/98	31.4	28.8	29.9	0.752
4/25/98	m578	RN	UAB 29	6/14/98	51 days	5/12/98-5/28/98	31.0	28.9	29.9	0.618
4/25/98	m591	RN	UAB 30	6/15/98	52 days	5/12/98-5/29/98	31.7	28.8	30.0	0.825
4/29/98	pd224	PD	UAB 17	6/23/98	56 days	5/18/98-6/4/98	33.5	29.7	31.2	1.271
4/29/98	pd235	PD	UAB 18	6/22/98	55 days	5/17/98-6/4/98	34.2	30.0	31.7	1.450
4/29/98			UAB 19	6/22/98	55 days	5/17/98-6/4/98	34.1	30.2	31.6	1.254
5/19/98	m1311	RN	UAB 21	7/4/98	50 days	6/5/98-6/20/98	34.9	31.3	32.9	1.037
5/19/98	m1315	RN	UAB 31	7/4/98	50 days	6/5/98-6/20/98	34.3	31.1	32.5	0.897

LayDate	Nest #	Location	Data Logger	HatchDate	Inc.Duration	Mid 1/3 Dates	MaxT Mid1/3	MinT Mid1/3	AvgT Mid1/3	SD
5/19/98	m1317	RN	UAB 32	7/4/98	50 days	6/5/98-6/20/98	34.9	31.3	32.8	1.028
5/19/98	m1321	RN	UAB 33	7/4/98	50 days	6/5/98-6/20/98	33.9	31.0	32.2	0.792
5/19/98	m1325	RN	UAB 34	7/4/98	50 days	6/5/98-6/20/98	34.5	31.3	32.7	0.881
5/19/98	m1335	RN	UAB 35	7/4/98	50 days	6/5/98-6/20/98	34.7	31.2	32.7	1.009
5/30/98	m1595	RN	#234-bottom	7/15/98	47 days	6/15/98-6/29/98	34.4	31.8	33.1	0.892
5/30/98	m1595	RN	#235-middle	7/15/98	47 days	6/15/98-6/29/98	34.8	32.0	33.5	0.995
5/30/98	m1595	RN	#237-top	7/15/98	47 days	6/15/98-6/29/98	35.2	32.5	34.1	0.947
5/31/98	m1730	RN	UAB 36	7/14/98	45 days	6/15/98-6/29/98	35.2	31.9	33.7	1.126
5/31/98	m1738	RN	UAB 37	7/15/98	46 days	6/15/98-6/30/98	33.8	31.6	32.8	0.738
5/31/98	m1744	RN	UAB 38	7/14/98	45 days	6/15/98-6/29/98	35.3	32.1	33.9	1.034
5/31/98	m1760	RN	UAB 39	7/16/98	47 days	6/16/98-6/30/98	35.1	31.8	33.8	1.038
5/31/98	m1758	RN	UAB 40	7/16/98	47 days	6/16/98-6/30/98	34.7	31.6	33.5	0.976
5/31/98	m1785	RN	UAB 42	7/15/98	46 days	6/15/98-6/30/98	34.8	31.5	33.5	1.077
5/31/98	m1766	RN	UAB 43	7/16/98	47 days	6/16/98-6/30/98	34.5	32.1	33.5	0.767
5/31/98	m1755	RN	UAB 45	7/14/98	45 days	6/15/98-6/29/98	34.7	31.6	33.4	1.008
5/31/98	m1767	RN	UAB 46	7/17/98	48 days	6/16/98-7/1/98	35.2	32.2	33.9	0.827
5/31/98	m1761	RN	UAB 47	7/17/98	48 days	6/16/98-7/1/98	35.5	31.6	33.8	1.120
5/31/98	m1762	RN	UAB 48	7/15/98	46 days	6/15/98-6/30/98	34.3	31.5	33.2	1.022
5/31/98	m1775	RN	UAB 49	7/17/98	48 days	6/16/98-7/1/98	35.1	32.1	33.9	0.826
5/31/98	m1604	RN	#232-bottom	7/15/98	46 days	6/15/98-6/30/98	34.3	31.8	33.0	0.819
5/31/98	m1604	RN	#233-middle	7/15/98	46 days	6/15/98-6/30/98	34.9	32.2	33.5	0.854
5/31/98	m1604	RN	#236-top	7/15/98	46 days	6/15/98-6/30/98	34.6	32.4	33.6	0.638
5/31/98	m1604	RN	#238-adj	7/16/98	47 days	6/16/98-6/30/98	34.6	32.7	33.8	0.546
5/31/98	m1605	RN	#239-bottom	7/16/98	47 days	6/16/98-6/30/98	34.8	32.0	33.6	0.883
5/31/98	m1605	RN	#240-middle	7/16/98	47 days	6/16/98-6/30/98	35.5	32.6	34.3	0.921
5/31/98	m1605	RN	#241-top	7/16/98	47 days	6/16/98-6/30/98	35.3	32.8	34.4	0.752
5/31/98	m1605	RN	#242-adj	7/16/98	47 days	6/16/98-6/30/98	34.2	32.2	33.5	0.617
6/5/98	m1865	RN	UAB 52	7/21/98	47 days	6/21/98-7/5/98	34.7	33.1	33.2	0.434
6/6/98	m1879	RN	UAB 54	7/24/98	49 days	6/22/98-7/8/98	35.3	33.3	34.2	0.557
6/8/98	m1990	RN	#228	7/23/98	46 days	6/23/98-7/8/98	34.4	33.2	33.8	0.307
6/8/98	m1989	RN	#230	7/26/98	49 days	6/24/98-7/10/98	35.0	33.6	34.3	0.402
6/9/98	m2017	RN	#229	7/26/98	48 days	6/25/98-7/10/98	35.6	33.9	35.0	0.442
6/9/98	m2018	RN	#231	7/28/98	50 days	6/26/98-7/11/98	35.0	33.0	34.4	0.554
6/13/98	pd435	PD	UAB 60	8/1/98	49 days	6/30/98-7/16/98	34.4	30.3	33.0	0.651
6/13/98	pd434	PD	UAB 65	8/1/98	49 days	6/30/98-7/16/98	35.2	30.3	33.1	0.544
6/13/98	tp579	TP	UAB 68	8/1/98	49 days	6/30/98-7/16/98	36.9	32.3	34.3	0.660

LayDate	Nest #	Location	Data Logger	HatchDate	Inc.Duration	Mid 1/3 Dates	MaxT Mid1/3	MinT Mid1/3	AvgT Mid1/3	SD
6/13/98	pd431	PD	UAB 69	7/31/98	48 days	6/29/98-7/14/98	36.6	30.3	33.6	0.844
6/13/98	tp580	TP	UAB 71	8/4/98	52 days	7/1/98-7/18/98	36.1	32.3	34.2	0.480
6/13/98	tp581	TP	UAB76	8/4/98	53 days	7/1/98-7/17/98	36.9	31.9	34.2	0.657
6/13/98	pd423	PD	UAB58	7/29/98	47 days	6/29/98-7/13/98	34.4	31.4	33.0	0.326
6/14/98	tp?	TP?	UAB 59	7/31/98	47 days	6/30/98-7/14/98	36.1	31.9	33.4	0.530
6/14/98	pd433	PD	UAB 62	7/31/98	47 days	6/30/98-7/15/98	36.1	30.3	33.4	0.632
6/14/98	tp584	TP	UAB 63	8/1/98	48 days	6/30/98-7/15/98	35.7	31.4	33.6	0.642
6/14/98	pd430	PD	UAB 64	7/30/98	46 days	6/30/98-7/15/98	36.6	31.1	33.6	0.620
6/14/98	pd438·	PD	UAB 72	8/1/98	48 days	6/30/98-7/15/98	34.8	30.3	32.9	0.588
6/14/98	tp592	TP	UAB 74	8/1/98	48 days	6/30/98-7/15/98	36.1	32.7	34.2	0.454
6/14/98	tp583	TP	UAB75	8/4/98	52 days	7/1/98-7/17/98	35.2	31.9	33.4	0.409
6/14/98	tp587	TP	UAB70	7/31/98	48 days	6/30/98-7/14/98	34.8	31.4	33.1	0.565
6/14/98	pd436	PD	UAB73	7/30/98	47 days	6/30/98-7/14/98	34.4	30.7	32.9	0.546
6/15/98	pd437	PD	UAB 57	8/1/98	47 days	6/30/98-7/15/98	34.4	30.7	32.9	0.545
6/15/98	tp601	TP	UAB 61	8/1/98	48 days	7/1/98-7/16/98	34.4	30.7	32.5	0.555
6/15/98	tp598	TP	UAB 67	8/4/98	50 days	7/2/98-7/18/98	35.2	31.1	33.2	0.459
6/21/98	pd451	PD	UAB 66	8/5/98	45 days	7/6/98-7/20/98	34.8	31.1	33.6	0.408
6/24/98	pd458	PD	UAB77	8/10/98	48 days	7/10/98-7/25/98	34.8	31.1	33.7	0.483
6/26/98	pd470	PD	UAB78	8/11/98	47 days	7/12/98-7/27/98	34.4	31.9	33.3	0.364
6/28/98	pd475	PD	UAB81	8/12/98	46 days	7/13/98-7/27/98	33.9	31.1	32.8	0.481
6/30/98	tp626	TP	UAB55	est 8/18/98	est 50 days	7/17/98-8/3/98	33.9	31.4	33.0	0.295
7/1/98	tp627	TP	UAB53	est 8/19/98	est 50 days	7/18/98-8/3/98	34.4	31.1	32.9	0.491
7/10/98	tp	TP	UAB50	est 8/28/98	est 50 days	7/27/98-8/12/98	33.6	31.1	32.5	0.287
7/10/98	tp	TP	UAB51	est 8/28/98	est 50 days	7/27/98-8/12/98	34.4	31.4	33.2	0.406
7/12/98	pd507	PD	UAB85	8/30/98	50 days	7/29/98-8/14/98	34.4	29.1	32.6	0.676
7/13/98	tp	TP	UAB88	8/26/98	45 days	7/28/98-8/11/98	34.8	31.4	33.6	0.363
7/16/98	tp	TP	UAB87	8/30/98	45 days	7/31/98-814/98	33.9	31.9	33.2	0.307

APPENDIX B

Hatchling Blood Samples Collected during the 1998 Kemp's Ridley Sex Ratio Project

Legend For Data Spreadsheet:

Location: RN= Rancho Nuevo, PD = Playa Dos, TP = Tepehuajes

Samples = The number of hatchlings from which blood samples were obtained from each nest

Blood Samples: 1998 Kemp's Ridley Sex Ratio Project

No.	Nest No.	Location	Samples
1	423	PD	10
2	430	PD	10
3	431	PD	8
4	433	PD	5
5	436	PD	10
6	579	TP	10
7	587	TP	10
8	589	TP	10
9	601	RN	10
10	817	RN	10
11	882	RN	10
12	1311	RN	9
13	1321	RN	7
14	1325	RN	9
15	1335	RN	7
16	1595	RN	10
17	1604	RN	10
18	1605	RN	7
19	1730	RN	8
20	1738	RN	10
21	1744	RN	10
22	1755	RN	10
23	1760	RN	9
24	1761	RN	6
25	1762	RN	10
26	1766	RN	10
27	1767	RN	10
28	1785	RN	10
29	1865	RN	10
30	1879	RN	10
31	1989	RN	10
32	1990	RN	10
33	2017	RN	3